

Evaluating Skin Penetration of Active Substitute using Near-infrared Chemical Imaging (NIR-CI)



Introduction

Near infrared chemical imaging (NIR-CI) has established itself as a powerful technique for pharmaceutical applications such as comparative studies for troubleshooting and development situations. It is a non-destructive technique and the samples would be available for further testing combined with a high degree of flexibility with respect to sample size and shape.

In this study NIR-CI was used to evaluate the penetration of a topical cosmetic active/drug delivery system, Lipodisq[®] (Malvern Cosmeceutics Ltd). Lipodisq[®] particles are in the size range of 10 to 40nm diameter, considerably smaller than conventional carrier systems such as liposomes (typically 50 to >1000nm). The nano-dimensions of a Lipodisq[®] particle are believed to enhance diffusion into the skin resulting in improved topical drug delivery. Lipodisq[®] are believed to exist in either discoidal or spherical form. An active substitute (AS) was used to represent the active principal ingredient (API) to enable depth of penetration to be evaluated. A hydrogel patch, containing Lipodisq[®] active substitute solution, was applied to the forearm of the test subject for 30 minutes. The patch was then removed and any excess solution cleaned from the skin surface. Pieces of tape were then sequentially applied to the area of skin and lifted off, 16 pieces of tape were used in total, Figure 2 (samples provided by Steve Tongue and Andy Harper, Malvern Cosmeceutics Ltd). These pieces of tape were then imaged to see if it was possible to visualize the extent of the

AS progression through the skin layers.

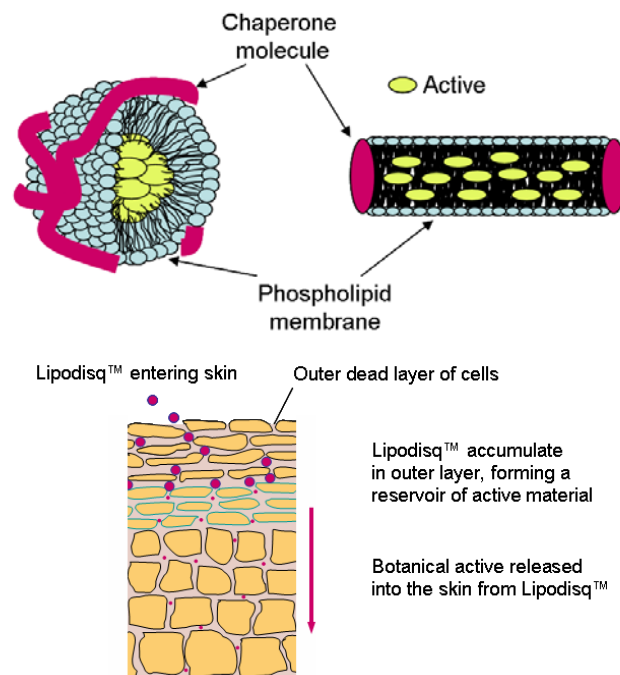


Figure 1: Lipodisq[®] possible structures and method of skin penetration



Figure 2: Photograph of the 16 tape lifts

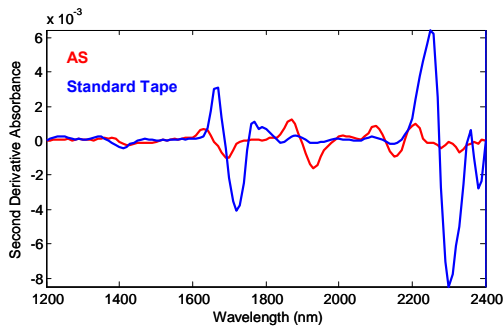
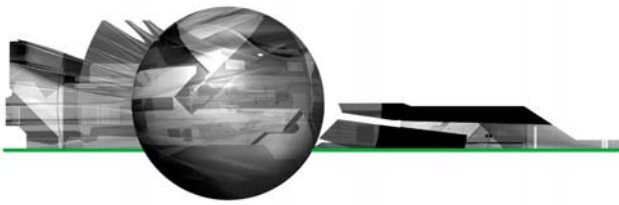


Figure 3: Reference spectra for sample components

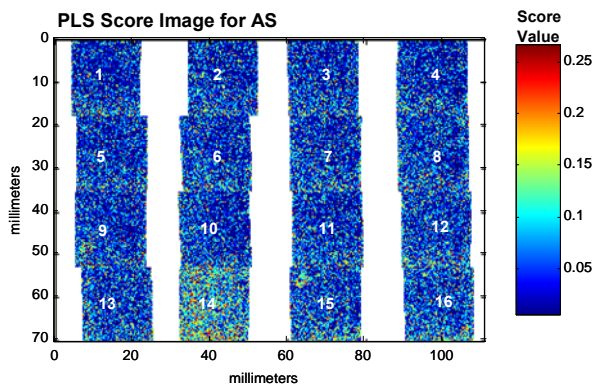


Figure 4: Concatenated AS score images for the 16 tape lifts

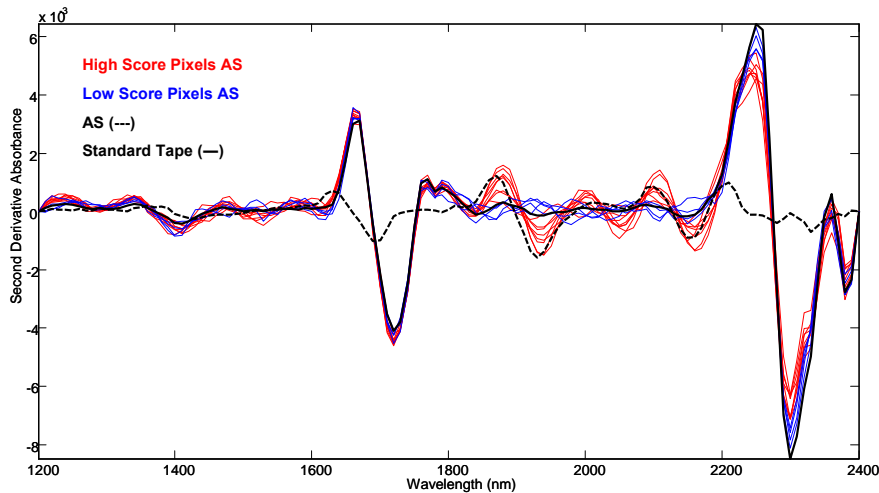


Figure 5: Overlay of reference component spectra with the high and low score AS spectra

NIR-CI Experimental

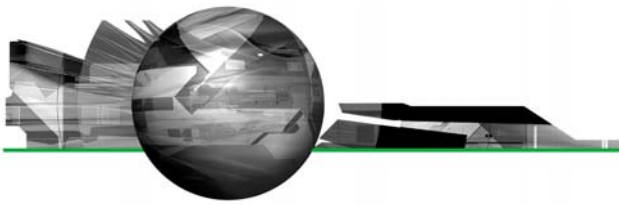
To collect NIR-CI data using the Malvern Instruments SyNIRgi, samples are first illuminated with broadband NIR light. After interaction with the sample, the resulting diffusely reflected light is collected with imaging optics. Wavelength selection is performed with a high resolution Liquid Crystal Tunable Filter (LCTF), and the resulting wavelength selected radiation (6 nm bandpass at 1600 nm) is focused onto a focal plane array with 320×256 pixels. A variety of imaging optics are available, to allow imaging of samples areas from ~ 3.2 mm x 2.5 mm ($10 \mu\text{m} \times 10 \mu\text{m}/\text{pixel}$) to 100×82 mm ($320 \mu\text{m} \times 320 \mu\text{m}/\text{pixel}$). The range covered in this study was 1200 – 2400 nm using the $80 \mu\text{m}/\text{pixel}$ objective. The pieces of tape were placed on top of the 100% reflectance standard to permit a transmittance measurement whereby the light passes through the sample twice to increase the possible sample signal available. The data collected are processed using ISys[®] software.

The data were corrected for background and dark measurements, converted to absorbance units, masked to remove non-sample regions then derivatized to remove physical contributions to the data.

Chemometric multivariate analysis based on a reference library, Figure 3, was applied to calculate the contribution of each relevant component at every pixel over the images. The contribution or abundance of each component in a pixel was given by a score value. A score value of 1 means that there is $\sim 100\%$ of that component present.

These score images were stuck together in the processing software so that they could be viewed on the same scale for direct comparison, Figure 4.

Initial inspection of the AS score images indicated that tape 14



appeared to contain more high score pixels than the others. In order to check that the chemometrics were identifying the correct regions as the AS, the spectra from the high and low score AS pixels were compared to the reference spectrum, Figure 5. This showed that the high score regions for the AS contained clear spectral

characteristics matching the reference AS spectrum compared to the low score regions that followed the tape reference to a greater extent.

Following this, the average images of the score files were calculated and are shown in Figure 6. This provides a value for the overall abundance in the

sample for each piece of tape and no 14 shows approximately double the value of the others.

As there is a spectrum for every pixel in the array it is possible to view the distribution of values over the image. For the case of the score images in Figure 4 the histograms represent the distribution of the AS over the pieces of tape, Figure 7. Statistical values are calculated that describe the distribution and these can provide quantitative metrics to help describe the images and can be used for further correlations. In this case the mean score values and the standard deviation of the histograms are shown, Table 1. The mean values are the same as from the averaged score images. The distribution for strip 14 is shifted to higher score values than the others which remain fairly similar. Strips 15 and 16 have been highlighted as containing the highest AS contributions next to strip 14.

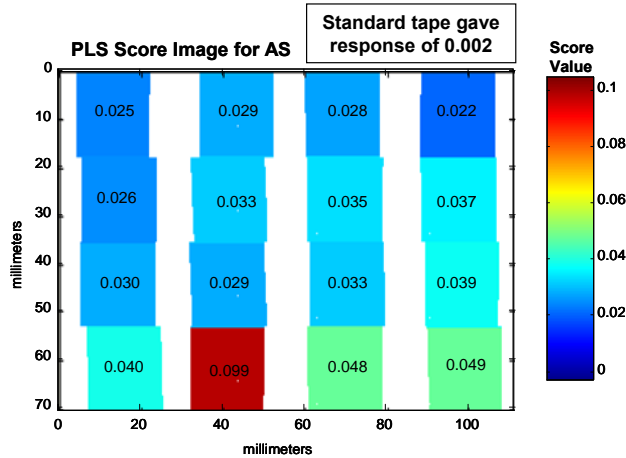


Figure 6: Concatenated averaged AS score images

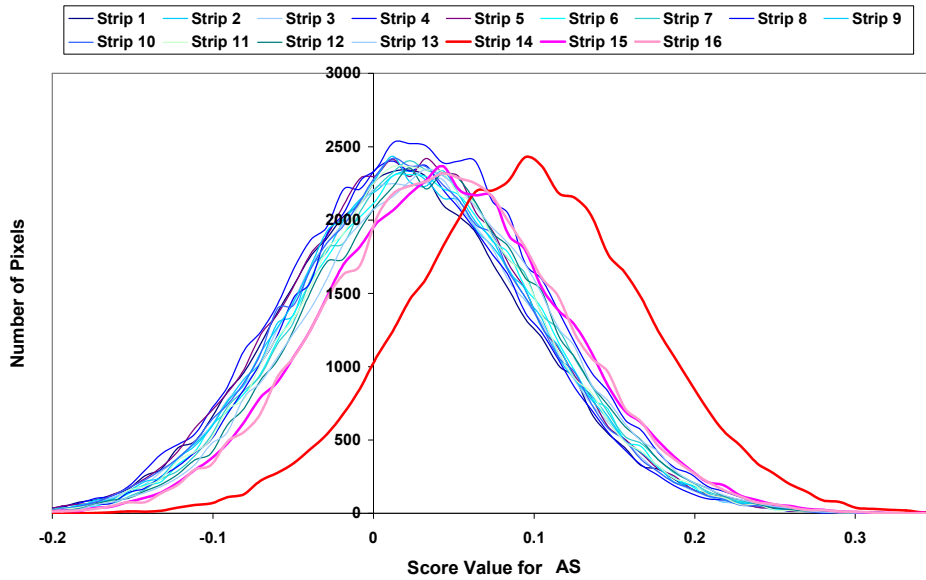
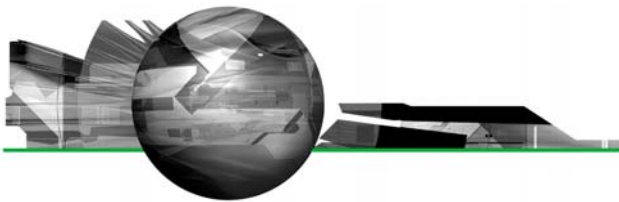


Figure 7: Overlay of the histograms from the AS score images



	Mean Value	Standard Deviation
Strip 1	0.025	0.077
Strip 2	0.029	0.076
Strip 3	0.028	0.076
Strip 4	0.022	0.076
Strip 5	0.026	0.076
Strip 6	0.033	0.076
Strip 7	0.035	0.076
Strip 8	0.037	0.076
Strip 9	0.030	0.076
Strip 10	0.029	0.076
Strip 11	0.033	0.075
Strip 12	0.039	0.075
Strip 13	0.040	0.075
Strip 14	0.099	0.074
Strip 15	0.048	0.076
Strip 16	0.049	0.075

Table 1: Sample statistics from histograms

Conclusions

The Malvern Instruments SyNIRgi near-infrared chemical imaging system provides rapid and non-destructive access to valuable information from a wide range of solid samples.

Considering the ease of use, the fast data collection time, the quantitative parameters and information that can be extracted from the data cubes, the SyNIRgi is a valuable addition to any laboratory performing pharmaceutical analysis.

In this study it was possible to identify variation in the AS contribution from tape strips representing progressively deeper layers of skin and hence provide an estimation of the depth of penetration. Values between ~2% to 10% AS abundance were observed, however the reference tape gave a ~0.2% contribution possible due to similarities in the spectra.

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